1967

Supplemental Vitamins in Feed and Water for Growing and Finishing Pigs and Growing Rats

Vernon L. Fritz

14

SUPPLEMENTAL VITAMINS IN FEED AND WATER FOR GROWING AND FINISHING PIGS AND GROWING RATS

BY

VERNON L. FRITZ

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in
Animal Science, South Dakota
State University

1967

SUPPLEMENTAL VITAMINS IN FEED AND WATER FOR GROWING AND FINISHING PIGS AND GROWING RATS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser _____ Date _____

Head, Animal Science Department _____ Date _____

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. Robert W. Seerley, Associate Professor of Animal Science, for his guidance and supervision throughout the study of this problem, and for his helpful suggestions during the writing of this manuscript. Grateful acknowledgment is also made to Dr. Richard C. Wahlstrom, Head of the Animal Science Department, for his constructive criticism of the final form of this thesis. Appreciation is also extended to other animal science staff members for assistance during the preparation of this thesis.

The author wishes to thank George Gastler, Associate Professor of Station Biochemistry, for conducting protein analysis on feed samples. A note of thanks is due to Dr. W. L. Tucker for his assistance with the statistical analysis.

The writer wishes to thank Mr. Roger Magstadt, Mr. Carl Ost and Mr. Chris Diepholz for their assistance in the care and management of the pigs.

Loving appreciation is extended to the author's wife, Pamela, for her patience, encouragement and assistance throughout this study. Gratitude is extended to Miss Marjorie Thom for typing the final copy of this manuscript.

VLF

TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDIX TABLES

INTRODUCTION

Vitamins are organic compounds required for normal growth and maintenance of animal life. Hopkins stated in 1906 that "no animal can live on a mixture of pure protein, fat and carbohydrate, and, even when the necessary inorganic material is carefully supplied, the animal still cannot flourish. The animal body is adjusted to live either upon plant tissue or other animals, and these contain countless substances other than protein, carbohydrate and fats." Hopkins called these substances accessory food factors.

McCollum and Davis (1913) gave the basis for vitamin classification. They proposed the names fat soluble A for the factor found in butter and water soluble B for the one concerned with beriberi as descriptive terms, since the first factor was extractable from food with fat solvents and the second factor was extractable with water. Vitamins are still classified as water and fat soluble.

Vitamins in swine diets have become more important in recent years as swine producers have changed to the confinement method of rearing pigs. When confined, swine are more likely to suffer from nutritional deficiencies, especially a deficiency of vitamins. However, with greater knowledge of nutrition and establishment of vitamin requirements, swine producers are now able to include the essential nutrients (carbohydrates, fats, proteins, minerals and vitamins) and feed additives in a completely mixed diet. This method of feeding swine has been widely accepted since completely mixed diets support excellent gains and feed utilization; however, this method of feeding is not

completely void of limitations. Addition of small amounts of micro-nutrients requires additional equipment in order to insure adequate distribution throughout the feed mixture. In addition, the ration is difficult to change once it is mixed.

Another potential method of feeding nutrients is through the drinking water. If this form of feeding proved sound and economical, then there may be advantages which do not exist in other forms of feeding swine. This method of supplying nutrients to swine has its greatest advantage in its flexibility. An automatic water line may be used to meter the proper dosage of nutrients to the animals. The addition of a small concentrated amount of vitamins to the drinking water requires no additional and perhaps less labor when compared to mixing vitamins in a carrier and then adding them to the diet. A daily supplementation is also possible and thus could permit the use of wormers and other medication through the same system. Environmental or physiological stresses often cause animals to stop eating, but animals seldom refuse water. Because animals will drink during sickness, therapeutic levels of vitamins and antibiotics can be and are commonly administered in the drinking water.

There may also be disadvantages of supplying vitamins in the water for swine. Pigs tend to waste water, especially during the warmer season. Some vitamins may be lost in wastage; however, water wastage can be minimized with proper waters. Variation in water consumption may present another problem. Water consumption will vary due to

season as well as individual needs, thus causing a range in vitamin intake from inadequate to more than adequate.

Although potentiality for this method of feeding swine exists, it must be made clear that this idea is based on an untried hypothesis. Therefore, an accurate evaluation of such a method cannot be made until further research has been completed. It was the purpose of this investigation to compare the performance of growing and finishing pigs given vitamins in the drinking water with pigs fed a completely mixed air-dry diet. For additional information on this method of feeding vitamins, growing male rats were fed a purified vitamin-free diet with supplemental vitamins in the drinking water and compared with the performance of rats fed a completely mixed purified diet.

REVIEW OF LITERATURE

Studies on Pigs' Requirement of Certain Vitamins

Vitamin A. Ellis (1946) reviewed the literature on the vitamin requirements of swine. The following three references were cited in Ellis' article. Morrison et al. (1920) and Nelson et al. (1922) found that pigs fed white maize did not grow as fast as pigs fed yellow maize and that a vitamin A supplement was needed for equivalent rate of gain. Golding and Foot (1935) noted that pigs maintained on a diet deficient in vitamin A or carotene had low liver vitamin A and carotene content.

Since the establishment of the need for vitamin A in swine diets, studies have been made to estimate the animals' requirement. Dunlop (1934) considered the vitamin A requirement of swine to be between 14 and 62 mg. of carotene per 100 lb. of ration. Guilbert et al. (1940) established a level of 18 to 24 I.U. of vitamin A per day per kg. of body weight as the requirement for growth, but three times the minimum level was required before adequate liver vitamin A was obtained. Braude et al. (1941) considered the minimum requirement to be 10 I.U. of vitamin A per lb. live weight per day for pigs 8 weeks of age and older.

Hentges et al. (1952) suggested that 25 ug. of purified carotene per kg. of body weight was the minimum daily requirement for depleted 8-week-old pigs to restore initial blood plasma vitamin A levels and to provide some vitamin A in the liver. Blood plasma vitamin A levels ranged from 14.2 to 23.8 ug. per 100 ml. of plasma while liver vitamin A

ranged from 0.13 to 0.39 ug. per gm. of liver when carotene was fed to pigs at a level of 25 ug. per kg. of body weight daily.

Frape et al. (1959) studied the vitamin A requirement of the pig by using offspring from sows with low vitamin A reserves. The minimum requirement of the young pig for a stabilized source of vitamin A palmitate on a dry carrier was 800 I.U. per lb. of feed. At this level average blood plasma vitamin A was 18.5 ug. per 100 ml. of plasma.

Myers et al. (1959) fed vitamin A to growing pigs at levels of 8, 16, 24 and 32 ug. of vitamin A per lb. of live weight. There was sufficient liver vitamin A in the 8 ug. level treatment group to indicate the level was adequate (316 ug. of vitamin A per gm. of fresh liver).

Smith et al. (1961) fed growing pigs a corn-soybean meal ration containing 0.54 mg. of carotene per lb. and supplemented it with different levels of vitamin A. Experimental results indicated that 400 I.U. of vitamin A per lb. of ration were adequate for maintaining optimum rate of gain and normal plasma vitamin A (33.9 ug. per 100 ml. plasma). Levels beyond 400 I.U. of vitamin A only tended to increase storage of vitamin A in the liver and did not significantly affect rate of gain. Liver vitamin A was 79.9 I.U. per gm. when pigs received 400 I.U. of vitamin A per lb. of ration. Hjarde et al. (1961) found that the vitamin A requirement of growing pigs was 20 I.U. per day per kg. of body weight for maintenance of the liver vitamin A depot.

Nelson et al. (1962) observed that 8 to 16 ug. of vitamin A per lb. of live weight per day produced normal plasma vitamin A, some liver

storage and low cerebrospinal fluid pressure in growing pigs. Blood vitamin A levels for the 8 and 16 ug. levels were 14.1 and 18.5 ug. per 100 ml. of plasma, respectively. Liver vitamin A for the same two treatment levels were 14.4 and 65.4 ug. per gm. of dry tissue, respectively.

Ullrey et al. (1965) studied the vitamin A and beta carotene requirement of the growing and finishing pig when depleted of vitamin A at a weight of 50 kg. Either 0.5 mg. of fermentation beta-carotene or 250 I.U. of vitamin A palmitate per kg. of ration supported maximum rate of gain; however, 3.5 mg. of fermentation beta-carotene or 1000 I.U. of vitamin A palmitate per kg. of diet was necessary to restore serum vitamin A concentration to predepletion levels.

Vitamin D. Johnson and Palmer (1939) found that pigs confined inside and having no direct sunshine did not store vitamin D and developed deficiency symptoms of the vitamin. A depletion of vitamin D resulted in a reduction of the blood plasma calcium from 10 to 12 mg. per 100 ml. of blood plasma to below 6 mg. per 100 ml. of blood plasma. Bethke et al. (1946) estimated the minimum vitamin D requirement for growing pigs to be 90 U.S.P. units per lb. of ration when the ration contained 0.6% calcium and 0.45% phosphorus.

Wahlstrom and Stolte (1958) investigated the need for vitamin D in swine rations for growing and finishing pigs. The addition of 90 U.S.P. units of vitamin D per lb. to rations containing 0.61% calcium and 0.48% phosphorus and complete in other dietary factors did not improve daily gain when compared to gains of pigs fed vitamin D-free

rations. However, when a low calcium ration (0.20% calcium and 0.37% phosphorus) was fed along with a free choice mineral supplement, growth rate was reduced significantly and five of eight pigs developed symptoms of rickets. Supplementing the ration with vitamin D increased gains and decreased the number of pigs with visible symptoms of rickets.

Luecke et al. (1961) studied the effect of supplementing vitamin D in swine rations containing 0.3, 0.5 or 0.7% calcium. The addition of 1000 I.U. of vitamin D per lb. of ration to each calcium level did not improve rate of gain and feed efficiency when compared to the addition of 100 I.U. of vitamin D per lb. of ration.

Miller et al. (1965) measured the effect of dietary vitamin D_2 level upon calcium, phosphorus and magnesium balance in baby pigs fed a purified casein-glucose diet. Mineral balance was measured by comparing level of dietary intake with fecal and urinary excretion. Pigs fed no dietary vitamin D excreted an excessive amount of calcium, phosphorus and magnesium in the fecal material but excreted less urinary calcium and magnesium. Retention of calcium, phosphorus and magnesium was greatly reduced in vitamin D_2 deficient pigs. A dietary vitamin D_2 level of 100 I.U. per kg. of diet produced a normal calcium, phosphorus and magnesium balance which was not improved by higher levels of vitamin D_2. In a subsequent report, Miller et al. (1965) evaluated the vitamin D_2 requirement of the baby pig when fed casein or soybean meal as the source of protein in the diets. Criteria checked were rate of gain, serum mineral level, skeletal development and mineral balance. The casein diet with 100 I.U. of vitamin D_2 per kg. of diet gave optimal

results for all criteria studied. However, only soybean meal diets containing 500 I.U. of vitamin D_2 per kg. of diet produced optimal rate of gain.

Thiamine. Ellis (1946) reviewed the literature on the vitamin requirements of swine. The following articles have been cited from Ellis' article. Hughes (1940) estimated the thiamine requirement for growing pigs to be 22 ug. per day per kg. live weight. Ellis and Madsen (1944) related the thiamine requirement to the carbohydrate, fat and protein intake since increasing the quantity of fat in the diet was found to reduce the needs for thiamine. The minimum requirement was determined to be 47 ug. per kg. live weight. However, this level did not enable the pig to store appreciable amounts of thiamine in the muscle tissues.

Heinemann et al. (1946) showed that thiamine can be stored by pigs and that pigs are able to draw on this reserve for metabolism. Miller et al. (1955) fed baby pigs diets containing 0, 0.5, 1.0, 1.5 and 2.0 mg. of thiamine per kg. of solids. The minimum thiamine requirement for optimum rate of gain and feed efficiency was 1.5 mg. per kg. of dietary solids intake.

Cunha (1957) reported a wide distribution of thiamine in feeds. Therefore, he concluded that a thiamine deficiency was not likely to occur in swine rations.

Riboflavin. Hughes (1939) showed that the lack of riboflavin in the diet caused symptoms of retarded growth, diarrhea and leg deformities in the pig. McMillen et al. (1949) studied the effect of liberal B vitamin supplementation on the growth of weanling pigs. Calcium pantothenate, riboflavin and nicotinic acid, when added to the basal ration, significantly increased rate of gain, improved feed efficiency by 25% and prevented the occurrence of deficiency symptoms.

Krider et al. (1949) determined that the minimum riboflavin requirement for the growing pig was 1.4 mg. per lb. of ration. Mitchell et al. (1950) showed that the riboflavin requirement for the growing pig was higher at a lower temperature. Requirements of 0.55 mg. of riboflavin per lb. of feed at 85° F. and 1.59 mg. per lb. of feed at 42° F. were established. Terrill et al. (1955) estimated that the riboflavin requirement for the growing pig was 0.41 to 0.65 mg. per lb. of diet when the environmental temperature was 53° F.

Niacin. Hughes (1943) studied the niacin requirement of the growing pig and he found that 5 to 10 mg. of niacin per 100 lb. of body weight were required for maximum rate of gain.

Luecke et al. (1947, 1948) reported the niacin requirement of pigs was influenced by protein and tryptophan content of the ration. When corn constituted a major portion of the ration, a niacin deficiency occurred. The addition of either 30 mg. of niacin per day per pig or 200 mg. of DL tryptophan alleviated the deficiency and gave a growth response.

Powick et al. (1948) and Cartwright et al. (1948) evaluated the niacin requirement of the pig. High protein diets and consequently high levels of tryptophan completely satisfied the niacin requirement of the pig. Braude et al. (1946) and Luecke et al. (1948) showed that the niacin requirement of the pig decreases as the pig grows older and heavier.

Becker et al. (1963) studied niacin-tryptophan relationships in the nutrition of the weanling pig. Weanling pigs needed approximately 6 mg. of available niacin per lb. of diet when the minimum tryptophan requirement was provided in the diet.

Pantothenic Acid. Hughes and Ittner (1942) estimated the daily pantothenic acid requirement of swine to be 179 to 268 ug. per kg. live weight; however, Ellis et al. (1943) used 515 ug. per kg. live weight to prevent nerve degeneration.

Luecke et al. (1950, 1953) observed apparent differences in requirement between individual pigs for pantothenic acid. The requirement for pantothenic acid for growing pigs ranged between 4.15 to 6.15 mg. per lb. of feed.

Catron et al. (1953) concluded that vitamin B_{12} and pantothenic acid exert a sparing action on each other if an adequate amount of one of the two is present in the diet. Pantothenic acid supplementation in the diet of the growing pig did not increase rate of gain when the ration contained an adequate amount of vitamin B_{12} and chlortetracycline.

Stothers et al. (1955) studied the pantothenic acid requirement of the baby pig. A level of 12.5 mg. calcium pantothenate per kg. of solids was necessary to produce optimum rate of gain and feed efficiency.

Barnhart et al. (1957) fed growing pigs a purified diet and supplemented it with six different levels of pantothenic acid (2, 3, 4, 5, 6 or 7 mg. per lb. of diet) with or without chlortetracycline. Results indicated no significant difference in rate of gain, daily feed consumption or feed required per pound of gain between the pigs fed the different ration treatments. There were no significant differences in hemoglobin content, red blood cell count, white blood cell count, differential white blood cell count, hematocrit value or clotting time between the pigs on the ration treatments.

Choline. Johnson and James (1948) established the fact that choline is needed by the baby pig. Pigs fed a synthetic milk diet (30% protein as casein) without supplemental choline had a slower rate of gain and developed fatty infiltration of the liver. Nesheim and Johnson (1950) studied the effect of a high level of methionine in the diet on the dietary choline requirement of the baby pig. Results indicated that the baby pig did not require dietary choline when the diet contained 1.6% methionine. Cunha (1957) explained that methionine can furnish methyl groups for choline synthesis. Choline, however, is effective only in sparing methionine which otherwise would be used to make up for the choline shortage. He further explained that methionine is not used up for choline synthesis if there is an adequate level of choline in the ration.

Gorodeckji (1963) studied the effect of adding choline to the diet of the young growing pig. The addition of 1 gm. of choline chloride per kg. of diet increased total gains and improved feed efficiency 27.2 and 6.0%, respectively, over the control pigs.

Vitamin B_{12}. Richardson et al. (1951), Vohs et al. (1951) and Catron et al. (1952) concluded that vitamin B_{12} increased rate of gain and improved feed efficiency when added to the ration for growing and finishing swine. Optimum performance was obtained with 4 to 5 ug. of vitamin B_{12} per lb. of ration.

Grifo et al. (1964) furnished further information on the vitamin B_{12} requirement of the growing and finishing pig when fed in dry lot or on pasture. All plant basal rations containing 0.36 to 1.27 mg. per ton of vitamin B_{12} activity were supplemented with 10, 20 or 50 mg. per ton of crystalline vitamin B_{12}. Rate of gain, feed consumption or feed efficiency was not improved by the addition of vitamin B_{12} to the basal ration. The author explains that a "carry over" effect from the dam's milk or from the animal protein in the creep feed may have caused a decreased need for dietary vitamin B_{12}.

Recommended Vitamin Requirements for Pigs and Rats

The National Research Council's committee on swine requirements has reviewed the literature on the requirements of various age pigs and they have established the minimum nutrient requirements. Table 1 shows the vitamin requirements for the various weight classes of pigs. The values are expressed as the minimum daily requirement.

TABLE 1. DAILY NUTRIENT REQUIREMENTS OF GROWING AND FINISHING PIGS

	Growing pigs			Finishing pigs (self-fed)					
				Meat type			Bacon type		
Live weight, lb.	10	25	50	100	150	200	100	150	200
Vitamins:									
Vitamin A, I.U.	640	1,200	1,280	2,120	2,720	3,200	2,080	2,600	2,840
Vitamin D, I.U.	80	180	288	318	408	480	312	390	426
Thiamine, mg.	0.48	1.0	1.6	2.6	3.4	4.0	2.6	3.2	3.6
Riboflavin, mg.	1.2	2.8	3.8	5.3	6.8	8.0	5.2	6.5	7.1
Niacin, mg.	8.0	16.0	19.2	26.5	34.0	40.0	26.0	32.5	35.5
Pantothenic acid, mg.	4.8	10.0	16.0	23.8	30.6	36.0	23.4	29.2	32.0
Pyridoxine, mg.	0.4	1.0	1.6	---	---	---	---	---	---
Choline, mg.	400	800	---	---	---	---	---	---	---
Vitamin B_{12}, mcg.	8.0	14.0	16.0	26.5	34.0	40.0	26.0	32.5	35.5

N.R.C. 1959. Nutrient Requirements of Domestic Animals, No. 2. Nutrient Requirements of Swine. National Research Council, Washington, D. C.

Recommended dietary vitamin levels for the growing rat were taken from Farris and Griffith (1962). These authors indicated that the growing rat needs a daily supply of the following vitamins: vitamin A, 0.3 ug.; thiamine, 10 ug.; riboflavin, 40 ug.; pyridoxine, 10 ug.; choline, 3 mg. and vitamin E, 1 mg. of alpha-tocopherol.

Storage of Vitamin A

Moore (1957) stated that the quantity of vitamin A accumulated and stored in the liver by mammals varies widely between species and also between individuals within the species. He further stated that age, sex and seasonal variation are factors that influence liver vitamin A content, but that the most important factor was the food supply. Experimental results indicated that it was possible to maintain rats in good health with liver reserves between 0 and 10,000 I.U. of vitamin A per gm. of liver simply by varying the dietary intake of vitamin A. Clayton and Baumann (1944) found that the hepatic storage of vitamin A appeared to be relatively independent of other biochemical processes taking place in the liver of the rat.

Lemley et al. (1947) showed that liver vitamin A may indicate both the state of nutrition of the animal and the availability of vitamin A when administered under different conditions. The storage of vitamin A in the livers of rats was investigated following oral, subcutaneous or intramuscular administration. The oral method was found to be most effective in producing good storage of vitamin A in the liver. Vitamin A injected subcutaneously was approximately 35% as

efficient over a 5-day period as the same amount given orally, while intramuscular injection was only 2% as effective as the oral method.

Johnson and Baumann (1947) investigated the storage and distribution of vitamin A in rats fed certain isomers of carotene. At a low level of carotene, 35 I.U. per day, more vitamin A appeared in the kidney than in the liver; at higher levels of intake more vitamin A appeared in the liver than in the kidney. In a subsequent report, Johnson and Baumann (1948) showed that rats gaining slowly, due to an insufficient feed intake, had more liver vitamin A than rats who ate more feed and gained faster. Guerrant (1949) observed that the amount and concentration of vitamin A in the liver increased rapidly throughout the period of rapid growth of weanling rats and reached a maximum concentration when the rat was 170 days old. Additional vitamin A was stored beyond this age, but the concentration of vitamin A per gm. of liver remained fairly constant. Male rats on balanced diets at 21, 37, 51, 73, 117 and 170 days of age had liver vitamin A concentrations of 50, 61, 95, 184, 655 and 1862 I.U. of vitamin A per gm. of liver, respectively.

Almquist (1952) indicated that the liver vitamin A is a source for the plasma vitamin A. Ganguly (1960) stated that the vitamin A content of the blood is tenaciously preserved even at the expense of the last traces of the liver vitamin A so that on prolonged depletion, although vitamin A may not be present in the liver, it can still be detected in the blood. However, without a dietary intake of vitamin A the blood also becomes depleted of vitamin A. This work is in agreement

with Glover et al. (1947), Ganguly and Krinsky (1953) and High and Wilson (1956).

Water Dispersible Vitamin A

Sorbel et al. (1952) indicated that fat-soluble vitamins have to be converted to water-soluble forms by means of derivatives or dispersing agents for absorption and transportation. Because of this problem, the author further stated the deficiencies of the fat-soluble vitamins on adequate intake occur frequently due to poor absorption or transportation in the body.

Halpern et al. (1947) compared vitamin A in the form of a water emulsion with vitamin A in oil when fed to chickens. The basal diet supplied all the necessary nutrients including vitamins in adequate amounts with the exception of vitamin A. All groups of chickens receiving the vitamin A in the form of a water emulsion had faster daily gains than those fed the vitamin A in oil.

Sorbel et al. (1948) administered vitamin A to young rats in oily and aqueous media. Liver storage was three times as great in groups fed the unsaponifiable fraction of fish liver oil dispersed in water as compared with the same fraction administered in maize oil. In subsequent work, Sorbel et al. (1949, 1950) fed postpartum mother rats vitamin A in aqueous dispersion and in oily solution. Suckling rats whose dams were given vitamin A in an aqueous solution had higher liver vitamin A which appeared to be due to the increased total secretion of vitamin A in the milk.

Squibb et al. (1956) depleted New Hampshire chicks of their vitamin A reserve in order to determine absorption of vitamin A in aqueous and oil solutions. When 7,500 I.U. of vitamin A were injected intramuscularly, the blood serum level for the aqueous solution after a 24-hour period was 66 ug. per 100 ml. When vitamin A in oil solution was used, absorption proceeded at a slower rate. The average serum level was only 29 ug. per 100 ml. after a 7-day period.

Christensen et al. (1958) conducted studies with rats and pigs on the absorption of vitamin A in solution in arachis oil or ethyl oleate or dispersed in water. A single dose of 3,000 I.U. of vitamin A was given orally or by intramuscular injection to rats weighing 150 gm. Vitamin A in aqueous dispersion administered orally to rats increased liver vitamin A content to 800 I.U. per gm. at 2 days, but the vitamin A level fell rapidly after 6 days. Vitamin A in oil increased the liver vitamin A content to 300 I.U. of vitamin A per gm. in 4 days. Immediately after injection of vitamin A in aqueous dispersion 90% was recovered in muscle tissue, whereas only 50% of the vitamin A in oil could be recovered immediately. However, muscle vitamin A remained constant after 21 days when vitamin A was administered in oil in contrast to complete absorption of the vitamin A in aqueous dispersion after 7 days. Pigs 8 days old were given 100,000 I.U. of vitamin A in a single dose. Vitamin A in an aqueous dispersion given orally or by intramuscular injection increased liver vitamin A to 1,200 I.U. of vitamin A per gm. of liver; vitamin A in oil by mouth caused only a small increase in liver vitamin A and the intramuscular injection of

vitamin A in oil did not increase the storage of vitamin A in the liver.

Wise et al. (1958) compared different methods of dispersing vitamin A in milk for dairy calves by placing vitamin A in an oil medium, in a homogenized emulsion or in an aqueous dispersion. Emulsification of oily supplements in milk did not noticeably improve absorption of vitamin A. Vitamin A in an aqueous dispersion, however, passed more quickly into the blood and gave a higher maximum level than either the oil solution or the emulsion.

Kieckebush (1960) gave vitamin A depleted rats 1.5 or 2.5 I.U. of vitamin A daily by pipette as plamitate in water or acetate of vitamin A free arachis oil. At a level of 1.5 I.U. daily, which is the amount required by the rat, vitamin A palmitate was significantly more effective than the acetate; at 2.5 I.U. of vitamin A daily the difference was smaller.

MATERIALS AND METHODS

Two experiments were conducted in this study. The first experiment consisted of four trials with growing-finishing pigs. The second experiment consisted of three trials with growing male rats. The trials were progressive in nature in that information obtained from the previous trial was utilized in planning all subsequent experimental work.

Experiment 1

Growing pigs weighing between 40 and 60 lb. were used in all four trials. The pigs were allotted on the basis of breed, genetic relationship, weight and sex. The sleeping houses for each trial were divided into equal lots with an adjoining concrete slab. Facilities for each lot included an automatic 80 gal. waterer and a six-cup self-feeder. All pigs were fed and watered ad libitum. Feeders and waterers were located inside during the colder months and outside during the warmer months; however, the pigs had access to the outside at all times.

All pigs received the same basal growing and finishing ration with the exception of lot 3 in trial 1 and lot 4 in trial 2. Lot 3, trial 1, was fed a mixture of corn and soybean meal and a mineral supplement free choice. Lot 4, trial 2, was fed shelled corn and a protein supplement free choice (table 2). All other pigs were fed a 16% protein grower ration (table 2) until the lot averaged 110 lb. and a 12% protein finishing ration was fed through the finishing period. The main treatment variable was the method of feeding vitamins. Positive

TABLE 2. COMPOSITION OF DIETS USED IN GROWING-FINISHING TRIALS

Item	Grower	Finisher	Free choice supplement	Free choice mineral
	%	%	%	%
Shelled corn	81.6	90.5	---	---
Soybean meal (50%)	14.0	5.5	64.0	---
Meat and bone scraps (50%)	2.5	2.5	20.0	---
Dehydrated alfalfa meal	---	---	10.0	---
Dicalcium phosphate	0.9	0.6	3.2	40.0
Limestone	0.4	0.3	0.3	40.0
Trace mineralized salt[a]	0.5	0.5	2.5	20.0
Vitamin-antibiotic premix[b]	0.1	0.1	---	---
Total	100.0	100.0	100.0	100.0
Calculated analysis				
Crude protein, %	15.7	12.2	44.1	---
Calcium, %	0.67	0.54	3.3	24.8
Phosphorus, %	0.57	0.50	1.7	7.4

[a] Trace mineral content in the salt mixture was 0.58% manganese, 0.015% cobalt, 0.08% copper, 0.8% zinc, 0.3% iron and 0.016% iodine.

[b] Vitamin-antibiotic premix provided 1,135 I.U. of vitamin A, 340 I.U. of vitamin D, 2 mg. of riboflavin, 4 mg. of pantothenic acid, 9 mg. of niacin, 10 mg. of choline chloride and 5 ug. of vitamin B_{12} per lb. of ration. Fifty milligrams of chlortetracycline, 50 mg. of sulfamethazine and 25 mg. of penicillin per lb. of ration were fed to 75 lb. body weight, thereafter 10 mg. of chlortetracycline per lb. of ration was fed. Hygromycin B was fed in the feed for 8 weeks (6 mg. per lb.).

control pigs were fed vitamins in the feed. Negative control pigs in trials 3 and 4 were not fed supplemental vitamins. All other pigs received their supplemental vitamins in the drinking water at low, medium or high levels. The medium level provided twice the quantity of vitamins per gallon of water as the low level and the high level provided four times the quantity of vitamins per gallon of water as the low level. It should be noted that the vitamin premix added to the feed differed in source from the vitamins added to the water. The vitamins added to the

feed were mixed with a carrier, whereas the vitamins added to the water were a purified, water-dispersible form. A fresh supply of water and supplemental vitamins were added approximately every third day. Vitamin intake was determined by the number of gallons of water the animals consumed, since the amount of vitamins added per gallon remained constant throughout the experimental period.

The pigs were weighed individually when allotted to the experiment and every 2 weeks thereafter. As they approached 200 lb. in weight they were weighed weekly and when the lot averaged 205 lb. they were removed from the experiment. Animals that died or were removed before the experiment was completed were autopsied and examined for gross pathological lesions. Accurate feed and water consumption records were kept throughout the trials.

At the termination of trials 3 and 4, feed and water were withheld from all barrows for a 6-hour period. A 40 ml. blood sample was drawn from the anterior vena cava for vitamin A determination. The blood samples were then centrifuged and the plasma was drawn off and frozen for analysis at a later date. The barrows were then slaughtered at a nearby packing plant so that liver samples could be obtained. The livers were collected on the kill floor and put in plastic bags with individual identification. They were frozen and later analyzed for vitamin A content.

Trial 1. Twenty-four Duroc growing pigs were allotted into three treatment groups of eight pigs per group. Each treatment lot contained four barrows and four gilts. Pigs in lot 1 were fed complete

mixed growing and finishing rations (table 2). Pigs in lot 2 were fed the same rations as lot 1, but the supplemental vitamins and antibiotic were added to the drinking water. A mixture of corn and soybean meal and a mineral supplement were fed free choice in lot 3. Vitamins and an antibiotic were added to the drinking water. The corn-soybean meal mixture was 16% crude protein and the mineral mixture consisted of 40% ground limestone, 40% dicalcium phosphorus and 20% trace mineralized salt (table 2). The vitamin-antibiotic mixture provided 2,560 I.U. of vitamin A, 576 I.U. of vitamin D, 3.2 mg. of thiamine hydrochloride, 7.6 mg. of riboflavin, 38.4 mg. of niacin, 32 mg. of calcium pantothenate, 800 mg. of choline chloride and 32 ug. of vitamin B_{12} per gallon of water. For ease of mixing 549 mg. of sugar were added with the vitamins to make a total of 1 gm. of premix added per gallon of water. Tylosin was added in every other 50 gallons of water at the rate of 0.8 mg. per gallon. The experimental plan was to include enough vitamins in the drinking water to meet their daily requirements. Criteria studied were rate of gain, feed efficiency, feed and water consumption.

Trial 2. Forty weanling Duroc and Hampshire pigs were allotted into four treatment groups. Pigs in lot 1 served as the control group and they were fed a complete mixed basal ration. The same rations were fed in lot 2 as lot 1, but the supplemental vitamins and antibiotic were added to the drinking water. The same ration was also used in lot 3 except a commercial vitamin-antibiotic mixture (Tylocine) was added to the drinking water. Pigs in lot 4 received shelled corn and a protein

supplement fed free choice and vitamins and antibiotic in the drinking water. The vitamins and antibiotic used in lots 2 and 4 were the same as used in trial 1 with the exception that the sugar was omitted. The addition of sugar permitted extensive fungus growth, which caused the solution to coagulate and clog the waterers. Elimination of the sugar from the premix greatly minimized fungus growth in the water tank reservoir. Tylocine was added to the water to provide 4,800 I.U. of vitamin A palmitate, 600 I.U. of vitamin D, 1.2 I.U. of vitamin E, 3.2 mg. of thiamine mononitrate, 24 mg. of D-pantothenic acid, 24 ug. of vitamin B_{12} and 0.96 mg. of folic acid per gallon of water. The complete mixed rations were the same as used in trial 1 (table 2). The free choice supplement is also shown in table 2. Criteria for measurement of response included rate of gain, feed efficiency, feed and water consumption.

Trials 3 and 4. These trials were designed to measure pig performance when three different levels of vitamins were added to the drinking water. Criteria for measurement of response included rate of gain, feed efficiency, feed and water consumption and liver and blood vitamin A content.

Forty Duroc growing pigs were allotted into five treatment groups in trial 3 and 20 Duroc and 20 Yorkshire pigs were allotted into five groups in trial 4. Each treatment group contained four barrows and four gilts. Pigs in lot 1 served as the negative control and were fed a corn, soybean meal, mineral ration. The supplemental vitamins were omitted from both the feed and the drinking water. Pigs in lot 2 were

fed complete mixed rations and served as the positive control group (diet with normal vitamin fortification). The same ration that was used for pigs in lot 1 was used for pigs in lots 3, 4 and 5, but low, medium and high levels of vitamins were added to the drinking water, respectively. The low level of vitamins in the water furnished 1,280 I.U. of vitamin A, 280 I.U. of vitamin D, 3.8 mg. of riboflavin, 1.6 mg. of thiamine, 19 mg. of niacin, 16 mg. of pantothenic acid, 400 mg. of choline chloride and 16 ug. of vitamin B_{12} per gallon of water. The medium level supplied twice and the high level four times that quantity of vitamins per gallon of water.

Procedure for vitamin A analysis was as follows: The preparation of a standard vitamin A curve was done according to A.O.A.C. methods (1960). Liver and blood plasma vitamin A was determined by the procedure outlined by Johnson and Baumann (1947). A Bausch and Lomb Spectronic 20 spectrophotometer rather than an Evelyn colorimeter was used to determine vitamin A concentration. Unless stated otherwise each sample was analyzed in duplicate.

Preparation of Sample

A. Liver

Approximately 30 gm. of pig liver were placed in a Waring blender and blended until the liver became a paste. From this amount a 5 gm. sample was taken for each vitamin A determination. The entire rat liver was cut with a scissors and a 2.5 gm. sample was used for each vitamin A determination.

Saponification

A. Liver

The minced liver was placed in a 200 ml. beaker and 35 ml. of 8% potassium hydroxide were added to the beaker. The contents were stirred several times and allowed to stand overnight at room temperature.

B. Blood

Five ml. of blood plasma were placed in a 100 ml. beaker. Five ml. of potassium hydroxide were added and allowed to stand for 15 minutes at room temperature. The remainder of the procedure was the same for both blood plasma and liver vitamin A determinations.

Extraction

Thirty-five ml. of ethanol (95 to 100%) were added to the sample and the contents were stirred vigorously. The sample was transferred to a 250 ml. separatory funnel. The beaker was rinsed with distilled water and the contents were added to the funnel.

The sample was extracted three times with 50 ml. of high grade hexane per extraction. This volume was reduced to 25 ml. per extraction. The organic phase (top layer) was pipetted off of the first two extractions. The inorganic phase (bottom layer) was drawn off for the last extraction. The other two aliquots of the organic phase were then added back into the separatory funnel. If an emulsion had developed in the organic phase due to excessive agitation, it was

allowed to stand for ten minutes. If the emulsion still persisted, a small amount of ethanol (5 to 10 ml.) was added.

The organic phase was washed four times with cold tap water (300 to 500 ml. per washing). The first two times the water was just poured in and drained off. The last two times the funnel was shaken and the water was drained off. This process removed the majority of soap produced from saponification. Excess water was removed from the material by adding 50 ml. of saturated salt solution (NaCl). The water phase was drawn off, leaving the clear, vitamin A absorbed solvent.

Solvent Removal

The flask containing the sample was transferred to a desiccator and evaporated to dryness under reduced pressure in a water bath (60° C.). It was not necessary to dry down the entire extract. The amount evaporated to dryness depended upon the amount of vitamin A in the liver. It was necessary to obtain a transmittancy reading of 20 to 80%. For the pig liver vitamin A determination 2.5, 5.0 or 10.0 ml. of the 150 ml. extract were placed under vacuum. For the pig blood and rat liver vitamin A analysis 25 ml. (one-third of the total extraction) were evaporated to dryness.

Reading of the Unknown

The residue was taken up in 1 ml. of moisture-free chloroform. One drop of acetic anhydride was added to remove any traces of moisture still present in the residue. The contents were transferred to a Spectronic 20 test tube. By rapid delivery, 4 ml. of a 20% solution of antimony trichloride in chloroform were added to the test tube and

read against a blank of 1 ml. of chloroform plus 4 ml. of the antimony trichloride solution at G_{620}.

Calculation of Vitamin A Concentration

The percent transmittance readings were converted to optical density by the use of a conversion table (L = 2-log G). These figures were then plotted against the standard vitamin A curve. One I.U. was equal to 0.3 ug. of vitamin A.

Experiment 2

A total of 156 Sprague Dawley white male rats weighing approximately 75 gm. each were used in three separate trials to evaluate the method of feeding vitamins in the water. The objective was to add vitamins in water at three different levels and compare these levels when fed to rats. Diets also varied in vitamin content to determine the effect of the vitamins in water when fed with diets of different vitamin content. These levels in both the feed and water were used to make all possible comparisons in performance.

Each trial was conducted for a period of 4 weeks. The rats were individually fed and watered ad libitum. The rats were assigned at random to the treatments and caged in a 60 cage battery which was located in a 70° F. air-conditioned room. Initial, 2 week and final weights were taken for each rat.

Table 3 shows the basal diet fed to all rats. The diet for each feed treatment differed only in its vitamin content and each diet was designated by the following codes: (0) no vitamins, (L) low level, (M) medium level and (H) high level of vitamins. The low level was

TABLE 3. RAT DIET

Ingredients	%
Corn starch	71
Casein	20
Corn oil	5
Salts[a]	4

[a] Contained 6.8% calcium carbonate, 30.8% calcium citrate, 11.3% calcium biphosphate, 3.5% magnesium carbonate, 3.8% magnesium sulfate, 12.5% potassium chloride, 21.9% dibasic potassium phosphate, 7.7% sodium chloride, .007% cupric sulfate, 1.53% ferric ammonium citrate, .02% manganese sulfate, .009% ammonium alum, .004% potassium iodide and .05% sodium fluoride.

formulated to supply one-half the recommended vitamin requirements. The medium level was twice the low level and the high level was three times the low level in the feed. Trial 1, however, did not have the high level of vitamins in the feed as did trials 2 and 3. Records were kept of all feed consumed by each rat.

Vitamin intake was determined by the total milliliters of water the rats consumed since the amount of vitamins added per milliliter remained constant throughout the experimental period.

Trial 1. A 3 x 4 factorial design composed of three feed treatments and four water treatments was used in this trial. Sixty white male rats were utilized for the 12 treatments. Tables 3 and 4 show the 18% crude protein basal rat diet used and the quantities of vitamins added in the feed or water, respectively, at low levels of supplementation. One treatment group did not receive supplemental vitamins in the

TABLE 4. AMOUNT OF VITAMINS PER KG. OF FEED OR LITER OF WATER FOR RATS

	Feed[a]	Water[b]
	Trial 1	
Vitamin A, I.U.	62.5	8.568
Thiamine, mg.	0.454	0.285
Riboflavin, mg.	1.818	1.142
Pyridoxine, mg.	0.454	0.285
Choline chloride, mg.	136.000	85.689
Vitamin E, mg.[c]	90.800	---
	Trials 2 and 3	
Vitamin A, I.U.	62.5	17.136
Thiamine, mg.	0.454	0.570
Riboflavin, mg.	1.818	2.284
Pyridoxine, mg.	0.454	0.570
Choline chloride, mg.	136.000	171.378
Vitamin E, mg.[c]	90.800	---

[a] The medium level provided twice that level in the feed and the high level, which was used in trials 2 and 3, provided three times that level in the feed.

[b] The medium level was twice that quantity and the high level was four times that quantity per liter of water.

[c] Vitamin E was provided at a constant level in all diets.

feed or water and served as the negative control group. The low level of vitamins in the feed contained 62.5 I.U. of vitamin A, 0.454 mg. of thiamine, 1.818 mg. of riboflavin, 0.454 mg. of vitamin B_6, 136.0 mg. of choline chloride and 90.8 mg. of vitamin E per kg. of diet. Vitamin E was added at a constant level to all diets. The low level of vitamin supplementation in the water included 8.568 I.U. of vitamin A, 0.285 mg. of thiamine, 1.142 mg. of riboflavin, 0.285 mg. of vitamin B_6 and 85.689 mg. of choline chloride per liter of water. Higher levels were also used to provide twice that level in the feed and twice or four

times that level in the water. Criteria studied were total gain, feed efficiency, total feed and total water intake.

Trials 2 and 3. Trials 2 and 3 were 4 x 4 factorial designs with four feed and four water treatments. A total of 48 white male rats were used for the 16 treatments in each trial. The same basal diet used in trial 1 was used in these trials. Also, the quantity of vitamins added to the low and medium levels of feed remained the same. However, an additional higher level of vitamins (3 times the low level) was added to the feed, since increased gains were obtained when the rats received supplemental vitamins in the feed and water in trial 1. It was also concluded from trial 1 that the vitamin level in the water should be increased due to lower water consumption than anticipated. Therefore, the vitamin levels were doubled in comparison to trial 1. Table 4 shows the low level of vitamin supplementation in the water. The low level included 17.136 I.U. of vitamin A, 0.570 mg. of thiamine, 2.284 mg. of riboflavin, 0.570 mg. of vitamin B_6 and 171.378 mg. of choline chloride per liter of water. The medium level was twice that quantity and the high level was four times that quantity per liter of water.

Criteria studied for measurement of response included total gain, feed efficiency, total feed and total water intake and liver vitamin A content.

RESULTS AND DISCUSSION

Experiment 1

Trial 1. The results of this trial are shown in table 5. Average daily gain and feed per pound of gain for lots 1 and 2 were 1.93, 3.31 and 1.84, 3.19 lb., respectively. These differences were nonsignificant; however, pigs in lots 1 and 2 gained significantly faster than pigs in lot 3 ($P < .01$). Pigs in lot 3 that were fed a mixture of corn and soybean meal, a mineral mixture free choice and vitamins and tylosin in the water gained slowly and required more feed per pound of gain. These pigs were removed at a light weight because of their poor performance. Although all the essential nutrients were available, the pigs did not balance their diet on a free choice basis. Feed consumption was low and consumption of mineral was also low which may provide a partial explanation for their poor performance. Wahlstrom and Stolte (1958) found that pigs fed a low calcium ration along with a free choice mineral supplement gained slowly and developed symptoms of rickets.

Average daily intake of water, supplemental vitamin A and riboflavin received in the feed or water by the pigs are also shown in the table. These figures do not include the naturally occurring carotene and riboflavin contained in the swine diets. These figures are averages and are listed to provide a possible explanation for any difference in the results which may have been due to different vitamin intakes. Average daily intakes for the remainder of the vitamins have not been

TABLE 5. SUPPLEMENTAL VITAMINS AND ANTIBIOTIC IN DRINKING WATER, EXPERIMENT 1, TRIAL 1

Item	Control complete ration[a]	Vitamins and antibiotic in water[b]	Free choice[b]
Lot number	1	2	3
No. of pigs	8	8	8[c]
Av. initial wt., lb.	56.6	57.5	56.6
Av. final wt., lb.	211.4	210.6	167.7
Av. daily gain, lb.	1.93[e]	1.84[e]	1.20
Av. daily feed, lb.	6.40	5.89	4.74
Feed per lb. gain, lb.	3.31	3.19	3.76
Av. daily water, lb.	8.07	8.24	8.47
Av. daily supplemental vitamin A, I.U.[d]	7264	2532	2603
Av. daily supplemental riboflavin, mg.[d]	12.80	7.52	7.73

[a] Provided 1,135 I.U. of vitamin A, 340 I.U. of vitamin D, 2 mg. of riboflavin, 4 mg. of calcium pantothenate, 9 mg. of niacin, 10 mg. of choline chloride and 5 ug. of vitamin B_{12} per lb. of diet.

[b] Provided 2,560 I.U. of vitamin A, 576 I.U. of vitamin D, 3.2 mg. of thiamine hydrochloride, 7.6 mg. of riboflavin, 38.4 mg. of niacin, 32 mg. of calcium pantothenate, 800 mg. of choline chloride and 32 ug. of vitamin B_{12} per gallon of water. Tylosin was added in every other 50 gallons of water at the rate of .8 mg. per gallon of water.

[c] One pig was removed from the experiment at 130 lb. body weight due to a broken leg, but he was included in the data.

[d] Calculated from the average daily feed and water intake. This does not include the naturally occurring carotene and riboflavin available in the feed.

[e] Daily gain was significantly faster than pigs in lot 3 ($P<.01$).

shown since the same proportion of vitamins was added to each treatment.

It should be noted that pigs in lot 1 received nearly three times as much supplemental vitamin A per day and nearly twice as much riboflavin as did pigs in lots 2 and 3. Although there was little difference in performance between pigs in lots 1 and 2, vitamin intake was probably adequate for both groups. The National Research Council table of requirements suggests 450 I.U. of vitamin A per pound of feed. Supplemental vitamin A intake for lot 2 was 430 I.U. per lb. of feed plus that derived from carotene in the corn. Smith et al. (1961) indicated that 400 I.U. of vitamin A per lb. of ration were adequate for optimum rate of gain and normal blood plasma in growing pigs. Ullrey et al. (1962) found that 250 I.U. of vitamin A per lb. of ration supported maximum rate of gain when fed to vitamin A depleted finishing pigs. However, 454 I.U. per lb. of ration was necessary to restore serum vitamin A concentration to predepletion levels.

The supplemental riboflavin intake for pigs in lot 2 also appeared adequate. Average daily intake was 7.52 mg. per head daily. The National Research Council suggests a daily riboflavin intake of 8.0 mg. for pigs weighing 200 lb. Krider et al. (1949) stated that the minimum riboflavin requirement for the growing pig was 1.4 mg. per lb. of ration.

In this trial barrows gained significantly faster than gilts ($P < .01$). The statistical analyses for all pig and rat trials are shown in the appendix tables.

Trial 2. The results of this trial are shown in table 6. Average daily gain was not significantly different between the four treatment groups. Pigs in lot 2, which were fed the same vitamin premix in their water as pigs in lot 2 of trial 1, gained slightly slower than the control pigs which was similar to the performance of the pigs on the same treatment in trial 1. In this trial pigs fed vitamins in the water (lot 2) required more feed per pound of gain than the control pigs, whereas they required less in trial 1. Therefore, the average feed efficiency for the two methods was similar. Since pigs in lot 3 required about the same quantity of feed per unit of gain as the control pigs, there appeared to be little difference in feed utilization between the two methods of feeding vitamins.

As in trial 1, pigs fed the complete ration with vitamins consumed more vitamins than pigs fed vitamins in water. The small difference in performance between the two groups may be related to the difference in vitamin intake, although the vitamin intake of both groups should have been adequate.

The method of free choice feeding shelled corn and supplement proved satisfactory in this trial. The small differences of slower daily gain and improved feed efficiency were typical in comparison to complete mixed rations (Hutchinson et al., 1957; Young et al., 1959; Hoefer, 1963).

In trial 2, there was a significant difference in final weight between barrows and gilts. Barrows were 17.2 lb. heavier than gilts at the end of the trial ($P < .01$).

TABLE 6. SUPPLEMENTAL VITAMINS AND ANTIBIOTIC IN DRINKING WATER, EXPERIMENT 1, TRIAL 2

Item	Complete ration			Free choice ration
	Control[a]	Vitamins and antibiotic in water[b]	Commercial Tylocine with vitamins in water[c]	Free choice vitamins and antibiotic in water[b]
Lot number	1	2	3	4
No. of pigs	10	10	10	10
Av. initial wt., lb.	42.2	42.8	40.5	39.6
Av. final wt., lb.	202.0	199.0	205.0	195.7
Av. daily gain, lb.	1.55	1.50	1.59	1.47
Av. daily feed, lb.	5.00	5.21	5.10	4.68
Feed per lb. gain, lb.	3.22	3.46	3.20	3.17
Av. daily water, lb.	9.24	9.21	10.90	9.82
Av. daily supplemental vitamin A, I.U.[d]	5675	2830	6280	3017
Av. daily supplemental riboflavin, mg.	10.00	8.40	8.37	8.95

[a] Provided 1,135 I.U. of vitamin A, 340 I.U. of vitamin D, 2 mg. of riboflavin, 4 mg. of calcium pantothenate, 9 mg. of niacin, 10 mg. of choline chloride and 5 ug. of vitamin B_{12} per lb. of diet. Tylosin was added at the level of 10 mg. and 5 mg. per lb. of diet.

[b] Provided 2,560 I.U. of vitamin A, 576 I.U. of vitamin D, 3.2 mg. of thiamine hydrochloride, 7.6 mg. of riboflavin, 38.4 mg. of niacin, 32 mg. of calcium pantothenate, 800 mg. of choline chloride and 32 ug. of vitamin B_{12} per gallon of water. Tylosin was added in every other 50 gallons of water at the rate of .8 mg. per gallon.

[c] Tylocine was added to the water to provide 4,800 I.U. vitamin A palmitate, 600 I.U. vitamin A, 1.2 I.U. vitamin E, 3.2 mg. of thiamine mononitrate, 6.4 mg. of riboflavin, 38.4 mg. of niacin, 3.8 mg. of pyridoxine hydrochloride, 24 mg. D-pantothenic acid, 24 ug. vitamin B_{12} and .96 mg. folic acid per gallon of water. Tylosin activity was 1 gm. per gallon.

[d] Calculated from the average daily feed and water intake. This does not include the naturally occurring carotene and riboflavin available in the feed.

Trial 3. The results of this trial are shown in table 7. Average daily gain was not significantly different between methods of feeding or levels of vitamins fed. Pigs fed the medium level of vitamins in water (lot 4) gained about 6% faster than pigs in the other treatments and all other pigs gained essentially the same, including the positive and negative control groups. Vitamin fortification improved feed utilization for pigs in all treatments in comparison to feed utilization for pigs in lot 1 which received no supplemental vitamins in the feed or water. It was surprising to find pigs gain fast without any supplemental vitamins, however, previous research has indicated that pigs do not always respond to supplemental vitamins (Barnhart et al., 1957; Grifo et al., 1964). There were no gross symptoms of vitamin deficiency in these pigs; they appeared normal and healthy throughout the trial. Pigs in lot 4 were the most efficient which is in agreement with the performance for pigs fed the same level of vitamins in the water (lot 2) in trial 1 but disagrees with results in trial 2. Daily feed and water consumption were not affected by the treatments.

Pigs receiving the vitamin supplemental control diet (lot 2) had significantly higher liver vitamin A levels than any other treatment ($P < .01$). There was, however, no significant difference in the content of vitamin A in blood plasma. These data suggest that vitamin A, or conversion of carotene to vitamin A, may have been adequate in all treatments since the plasma level of vitamin A was relatively high in the vitamin-free treatment. It may also mean that vitamin A was

TABLE 7. VITAMINS IN WATER FOR GROWING-FINISHING PIGS, EXPERIMENT 1, TRIAL 3

Item	No vitamins added	Vitamins added in diet[a]	Level of vitamins in water		
			Low[b]	Medium	High
Lot number	1	2	3	4	5
No. pigs	7[c]	8	8	8	8
Av. initial wt., lb.	43.0	42.7	42.6	43.5	43.5
Av. final wt., lb.	203.0	202.5	202.5	203.0	202.3
Av. daily gain, lb.	1.69	1.68	1.68	1.80	1.71
Av. daily feed cons., lb.	5.77	5.47	5.58	5.59	5.74
Feed per lb. gain, lb.	3.45	3.26	3.32	3.11	3.36
Av. daily water, lb.	9.28	8.67	8.89	8.37	8.68
Av. daily supplemental vitamin A, I.U.[d]	---	6208	1366	2572	5335
Av. daily supplemental riboflavin, mg.[d]	---	10.94	4.05	7.63	15.83
Plasma vitamin A, mcg./100 ml. (least square mean)	34.5	31.1	19.1	21.5	22.5
Liver vitamin A, I.U./gm. (least square mean)	31.0	155.8[e]	29.6	49.8	75.5

[a] Provided 1,135 I.U. of vitamin A, 340 I.U. of vitamin D, 2 mg. of riboflavin, 4 mg. of calcium pantothenate, 9 mg. of niacin, 10 mg. of choline chloride, and 5 ug. of vitamin B_{12} per lb. of diet.

[b] Provided 1,280 I.U. of vitamin A, 280 I.U. of vitamin D, 3.8 mg. of riboflavin, 1.6 mg. of thiamine, 19 mg. of niacin, 16 mg. of pantothenic acid, 400 mg. of choline chloride, and 16 ug. of vitamin B_{12} per gallon of water. The medium level was twice that quantity and the high level four times that quantity per gallon of water.

[c] One pig died during the trial at 120 lb. body weight. An autopsy showed that death was due to hemorrhagic enteritis. Data on this treatment were adjusted to compensate for the loss of this pig.

[d] Calculated from the average daily feed and water intake. This does not include the naturally occurring carotene and riboflavin available in the feed.

[e] Positive control pigs had significantly higher liver vitamin A levels than all other treatments ($P < .01$).

mobilized to meet tissue requirements as reported by Glover et al. (1947), Almquist (1952), Ganguly and Krinsky (1953), High and Wilson (1956), Moore (1957) and Ganguly (1960). Vitamin A intake was approximately the same for pigs in lots 2 and 5, yet liver storage of vitamin A was much greater with the vitamins in the diet than in the water. Thus, these data suggest better storage of the vitamins in the feed or instability or settling out of the vitamins in the water.

As in the previous two trials, there was a significant difference in final weight between barrows and gilts. Barrows were 19.8 lb. heavier than gilts at the end of the trial ($P<.01$).

Trial 4. Trial 4 was a replicate of trial 3. The results are shown in table 8. Average daily gain and feed efficiency in this trial were essentially the same for all treatments including the negative and positive control groups. Pigs in lot 4 fed the medium level of vitamins in the water were again the most efficient. It is interesting to note that these pigs have been relatively efficient in feed conversion in three of four trials and that feed utilization was not as good with the higher level of vitamins in the water in both trials 3 and 4. Although there is insufficient evidence available here to make conclusions, the data suggest that the higher level of vitamins may have been detrimental to good feed efficiency.

Pigs in the positive control group (lot 2) did have significantly higher plasma vitamin A levels ($P<.05$) when compared to the other treatment groups. In addition, pigs in the control group had significantly higher stores of liver vitamin A ($P<.01$). The negative control

TABLE 8. VITAMINS IN WATER FOR GROWING-FINISHING PIGS, EXPERIMENT 1, TRIAL 4

Item	No vitamins added	Vitamins added in diet[a]	Level of vitamins in water		
			Low[b]	Medium	High
Lot number	1	2	3	4	5
No. pigs	8	8	8	8	8
Av. initial wt., lb.	52.8	58.9	55.0	53.4	56.8
Av. final wt., lb.	200.5	201.0	200.0	201.0	200.0
Av. daily gain, lb.	1.64	1.61	1.64	1.64	1.63
Av. daily feed cons., lb.	5.37	5.23	5.14	5.11	5.26
Feed per lb. gain, lb.	3.27	3.24	3.13	3.12	3.23
Av. daily water, lb.	8.57	8.13	9.07	9.90	8.22
Av. daily supplemental vitamin A, I.U.[c]	---	5936	1393	3042	5052
Av. daily supplemental riboflavin, mg.[c]	---	10.46	4.14	9.03	14.99
Plasma vitamin A, mcg./100 ml. (least square mean)	10.9	19.1[d]	13.5	12.1	13.1
Av. liver vitamin A, I.U./gm.	10.2	174.3[e]	12.8	16.6	43.3

[a] Provided 1,135 I.U. of vitamin A, 340 I.U. of vitamin D, 2 mg. of riboflavin, 4 mg. of calcium pantothenate, 9 mg. of niacin, 10 mg. of choline chloride, and 5 ug. of vitamin B_{12} per lb. of diet.

[b] Provided 1,280 I.U. of vitamin A, 280 I.U. of vitamin D, 3.8 mg. of riboflavin, 1.6 mg. of thiamine, 19 mg. of niacin, 16 mg. of pantothenic acid, 400 mg. of choline chloride and 16 ug. of vitamin B_{12} per gallon of water. The medium level was twice that quantity and the high level four times that quantity per gallon of water.

[c] Calculated from the average daily feed and water intake. This does not include the naturally occurring carotene and riboflavin available in the feed.

[d] Positive control pigs had significantly higher plasma vitamin A levels ($P<.05$).

[e] Positive control pigs had significantly higher liver vitamin A levels ($P<.01$).

pigs had the lowest average values of plasma and liver vitamin A content. Liver vitamin A content gradually increased with more vitamin A in the water, but the storage was not as high as storage with vitamin A in the feed, which was true in trial 3 also. Vitamin A intake was the highest in lot 2 also, which may have had some effect on the total content in the liver.

Experiment 2

Trial 1. The results of this trial are shown in tables 9 and 10. Three levels of vitamins in the feed, zero, low and medium, were fed with four vitamin levels in the water, zero, low, medium and high, to give 12 treatment groups (table 9). Rats fed the purified diet without any supplemental vitamins lost weight. Although they consumed approximately 50% of the intake of rats receiving the highest level of vitamins, they still lost weight. There was general improvement in total weight gain and feed efficiency with higher levels of vitamins in the water, but this was not consistent with the medium level of vitamins in the water. The rats did not grow as fast or as efficiently as rats fed the low level of vitamins in water with the feed containing no vitamins or the medium level of vitamins. The cause of this problem could not be determined and it did not occur in subsequent trials. Nevertheless, the level of vitamins in the water had an influence on the performance of the rats. The effect appeared to be greater, as expected, when fed with a vitamin-free diet than a diet with adequate vitamins.

TABLE 9. THREE LEVELS AND TWO METHODS OF FEEDING VITAMINS TO RATS, EXPERIMENT 2, TRIAL 1[a]

Treatment[b]		Av. total gain, gm.	Feed-gain ratio, gm.	Av. total vitamin A intake, I.U.	Av. total riboflavin intake, mg.	Av. total feed, gm.	Av. total water, ml.
Feed	Water						
0	0	- 2	---	---	---	175	275
0	L	20	10.71	3.1	0.40	213	363
0	M	8	25.30	5.6	0.74	194	326
0	H	66	4.05	13.6	1.81	269	396
L	0	51	4.99	16.0	0.47	256	343
L	L	72	3.87	20.8	0.94	280	381
L	M	80	3.74	26.8	1.62	298	473
L	H	111	3.07	37.5	2.77	343	469
M	0	101	3.14	39.6	1.15	317	492
M	L	126	2.88	49.2	1.84	362	460
M	M	96	3.33	47.4	2.15	320	431
M	H	117	3.10	61.4	3.47	362	472

[a] Five male rats per treatment, averaged 73.2 gm. each at the start and they were fed 4 weeks.
[b] 0 = no vitamins, L = low, M = medium, H = high level of vitamins.

Total feed intake was increased when more vitamins were fed. Water consumption also increased with the higher intake of feed and vitamins, but the difference was smaller between treatments than the effect on feed consumption.

Table 10 summarizes the results of this trial and compares the average performance of rats fed different levels of vitamins in the water with the performance of rats fed different levels of vitamins in the feed. There was a significant difference in total gain for rats fed different levels of vitamins in the water. Rats fed the high level of vitamins in the water had a significantly greater total gain than rats receiving no supplemental vitamins in the water ($P<.01$). They also had a greater total gain than rats fed the medium level of vitamins in the water ($P<.05$). Rats fed the low level of vitamins in the water had faster gains and more efficient feed utilization than rats receiving the medium level of vitamins in the water. These differences cannot be fully accounted for other than the fact that rats receiving the low level of vitamins had a greater feed intake which may have produced faster gains.

Rats fed the low and medium levels of vitamins in the feed had significantly greater total gains than rats receiving no supplemental vitamins in the feed ($P<.01$). The medium level of vitamins in the feed also improved feed efficiency for these rats over those receiving no supplemental vitamins in their diet ($P<.05$).

In general, average daily gain for rats increased with the increase in vitamins in the feed or water. The fastest gaining rats

TABLE 10. EFFECTS OF VITAMINS IN WATER VERSUS VITAMINS IN FEED FOR RATS, EXPERIMENT 2, TRIAL 1

	Vitamin level[a]	No. of rats	Av. total gain, gm.	Feed-gain ratio, gm.	Av. total vitamin A intake, I.U.	Av. total riboflavin intake, mg.	Av. total feed, gm.	Av. total water, ml.
Water	0	15	50	5.00	18.5	0.54	249	370
	L	15	73	3.93	24.4	1.07	285	401
	M	15	61	4.43	26.6	1.50	271	410
	H	15	98[b,c]	3.31	37.5	2.68	325	446
Feed	0	20	23	9.27	5.6	0.74	213	340
	L	20	79[b]	7.74	25.3	1.45	294	416
	M	20	110[b]	3.10[d]	49.4	2.75	340	464

[a] 0 = no vitamins, L = low, M = medium, H = high level of vitamins. However, each level of vitamins in the water includes all levels of vitamins from feed and each level of vitamins in the feed includes all levels of vitamins in the water.

[b] Significantly different than the (0) level of vitamins ($P < .01$).

[c] Significantly different than the medium (M) level of vitamins ($P < .05$).

[d] Significantly different than the (0) level of vitamins ($P < .05$).

were also the most efficient in feed utilization. There were no significant differences in total gain or feed utilization when the performance of rats fed vitamins in the feed was compared with the performance of rats fed the same level of vitamins in the water.

After the completion of trial 1, it was concluded that the vitamin level in the water should be increased due to lower water consumption by the rats than anticipated. Therefore, in trials 2 and 3 the vitamin levels in the water were doubled in comparison to trial 1. Also, an additional group of rats was fed a higher level of vitamins (3 times the low level) in the feed because of the faster gains of rats receiving vitamins in the feed and water in trial 1.

Trials 2 and 3. The individual treatment results of trials 2 and 3 are shown in tables 11 and 12, respectively, and a summary on the feed and water methods of feeding vitamins in both trials is shown in table 13. Total gain generally improved with each higher increment of vitamins in the water when fed with the vitamin-free diet and the low and medium level diets. The same pattern of improvement held true with the high vitamin diet in trial 3, but the pattern did not hold true in trial 2.

Feed efficiency improved in the same pattern as total gain improved. Both improvement in gain and feed utilization were related to a higher intake of vitamins in the water. The effect was smaller at the higher levels of intake when the rats were already receiving an adequate quantity of vitamins in the feed. In general, the liver

TABLE 11. FOUR LEVELS AND TWO METHODS OF FEEDING VITAMINS TO RATS, EXPERIMENT 2, TRIAL 2[a]

Treatment[b]		Av. total gain, gm.	Feed gain ratio, gm.	Av. liver vitamin A, I.U./gm.	Av. total vitamin A intake, I.U.	Av. total riboflavin intake, mg.	Av. total feed, gm.	Av. total water, ml.
Feed	Water							
0	0	-20	---	16.3[c]	---	---	178	298
0	L	58	4.13	8.9[c]	4.9	0.66	241	575
0	M	66	4.30	10.7	9.7	1.29	281	565
0	H	123	2.93	6.4	20.2	2.70	359	590
L	0	42	6.19	15.7	16.3	0.74	261	425
L	L	106	3.42	6.9	31.3	1.81	363	503
L	M	136	2.82	4.3	40.7	2.93	384	488
L	H	137	2.89	5.2	58.4	5.21	395	492
M	0	54	5.21	13.2	35.1	1.02	281	475
M	L	114	3.18	6.1	53.1	2.36	362	458
M	M	120	3.08	4.8[c]	61.2	3.33	371	433
M	H	132	2.87	5.0	80.5	5.80	379	483
H	0	125	3.02	3.3	70.9	2.06	378	543
H	L	111	3.20	8.2	74.3	2.94	356	438
H	M	104	3.26	5.4	79.0	3.90	339	450
H	H	135	2.87	4.0[c]	104.3	6.31	389	458

[a] Three male rats per treatment, averaged 81.7 gm. each at the start and they were fed 4 weeks.
[b] 0 = no vitamins, L = low, M = medium, H = high level of vitamins.
[c] Average of 2 rats.

TABLE 12. FOUR LEVELS AND TWO METHODS OF FEEDING VITAMINS TO RATS, EXPERIMENT 2, TRIAL 3[a]

Treatment[b]		Av. total gain,	Feed gain ratio,	Av. liver vitamin A,	Av. total vitamin A intake,	Av. total riboflavin intake,	Av. total feed,	Av. total water,
Feed	Water	gm.	gm.	I.U./gm.	I.U.	mg.	gm.	ml.
O	O	5	38.60	8.8	---	---	180	275
O	L	24	8.71	4.6	4.1	0.54	212	475
O	M	71	4.33	3.8	7.9	1.06	309	463
O	H	118	3.22	2.9	19.5	2.60	380	568
L	O	48	5.59	3.5	16.8	0.49	268	395
L	L	92	3.81	4.8	30.4	1.75	352	488
L	M	91	3.77	8.5	38.6	2.89	345	496
L	H	103	3.38	2.5	58.0	5.47	347	530
M	O	91	3.57	3.2	40.4	1.17	323	512
M	L	111	3.31	2.0	55.5	2.63	366	570
M	M	119	3.24	3.6	64.6	3.58	386	476
M	H	120	3.28	5.8	92.4	7.19	394	630
H	O	85	4.00	3.9	63.6	1.84	339	442
H	L	98	3.78	7.1	79.1	3.27	372	545
H	M	100	3.58	4.7	82.9	4.07	357	465
H	H	121	3.12	4.1	109.2	7.16	379	557

[a] Three male rats per treatment, averaged 75.6 gm. each at the start and they were fed 4 weeks.
[b] O = no vitamins, L = low, M = medium, H = high level of vitamins.

vitamin A levels were higher in the treatments without vitamins in the water, except with the high level of vitamins in the feed.

Table 13 compares the performance of rats fed vitamins in the water with the performance of rats fed vitamins in the feed. Average liver vitamin A levels for rats in trials 2 and 3 are also compared for the two methods of feeding.

The performance of rats in trials 2 and 3 coincide with the performance of rats in trial 1. As the level of vitamins increased in the feed or water, average total gain increased and feed required per unit of gain decreased. Rats receiving the medium and high levels of vitamins in the water had significantly ($P < .01$) greater total gains and more efficient feed utilization than rats receiving no supplemental vitamins in the water. Gaining ability and feed utilization of rats fed the medium and high levels of vitamins was also improved over the performance of rats fed the low level of vitamins in the water, but the differences were not significant. The high level of vitamins in the water produced greater and more efficient gains for rats than did the medium level of vitamins; however, these differences were not significant.

Rats receiving the medium and high levels of vitamins in the feed had significantly greater total gains than rats receiving no supplemental vitamins in the feed ($P < .05$). Feed utilization was also improved significantly ($P < .01$). The medium and high levels of vitamins also produced greater but nonsignificant gains and more efficient feed utilization than the low level of vitamins. The high

TABLE 13. EFFECTS OF VITAMINS IN WATER VERSUS VITAMINS IN FEED FOR RATS
EXPERIMENT 2, TRIALS 2 AND 3

	Vitamin level[a]	No. of rats	Av. total gain, gm.	Feed gain ratio, gm.	Av. liver vitamin A I.U./gm.[b]	Av. total vitamin A intake, I.U.	Av. total riboflavin intake, mg.	Av. total feed, gm.	Av. total water, ml.
Water	0	24	54	5.14	8.5	30.4	0.88	276	421
	L	24	89	3.67	6.1	41.6	2.00	328	506
	M	24	101[d]	3.42	5.7	48.1	2.88	346	480
	H	24	124[d]	3.05[d]	4.5[c]	67.8	5.30	378	538
Feed	0	24	56	4.92	7.9	8.3	1.10	268	476
	L	24	94	3.59	6.4	36.3	2.63	339	477
	M	24	108[c]	3.33[d]	5.5	60.4	3.38	358	505
	H	24	110[c]	3.30[d]	5.0	82.9	3.95	364	487

[a] 0 = no vitamins, L = low, M = medium, H = high level of vitamins.
[b] Least square means.
[c] Significantly different than the (0) level of vitamins ($P < .05$).
[d] Significantly different than the (0) level of vitamins ($P < .01$).

level of vitamins in the feed did not improve total gain or feed efficiency when compared to the medium level of vitamins in the feed.

Liver vitamin A was also used as criteria to measure performance of rats fed different levels of vitamins in the feed and water. Lemley et al. (1947) indicated that liver vitamin A may indicate both the state of nutrition of the animal and the availability of vitamin A when administered under different conditions. These results do not indicate any major differences in liver vitamin A levels due to the two methods of feeding. However, rats receiving the high level of vitamins in the water had significantly lower liver vitamin A levels than rats receiving no supplemental vitamins in the drinking water ($P<.05$). This same trend is also observed when the vitamins were supplied through the feed. These results are similar to those of Johnson and Baumann (1948) who reported that liver vitamin A was higher for rats whose rate of gain was hindered due to insufficient feed intake than the rats that gained faster.

In all three trials of experiment 2, there are interactions between feed and water levels that may have a tendency to affect the performance of the rats (tables 11, 12 and 13). Performance results in trials 2 and 3 also show significant differences between replicates. These factors make it difficult to draw or separate exact effects of each variable, but in general the results suggest that vitamins fed in the water to rats produce gains and feed utilization equivalent to the gains and feed utilization of rats receiving supplemental vitamins in the feed.

SUMMARY AND CONCLUSIONS

One pig experiment and one rat experiment were conducted to evaluate the performance of animals fed supplemental vitamins in the drinking water with vitamins fed in a completely mixed ration. The pig experiment consisted of four trials and the rat experiment had three trials.

The performance of pigs fed an adequate level of vitamins in the water was essentially the same as the performance of pigs fed the complete mixed control ration. In trial 1, pigs in lots 1 and 2 had similar rates of gain and their feed conversion was also similar. However, pigs in lot 3, trial 1, that were fed a mixture of corn and soybean meal, a mineral mixture free choice and vitamins and tylosin in the water gained significantly slower ($P < .01$) and required more feed per pound of gain. It appeared that feed and mineral consumption was too low to provide rapid gains. In this trial, barrows gained significantly faster than gilts ($P < .01$).

In trial 2, average daily gain was not significantly different between lots of pigs fed vitamins in the feed or water. Feed efficiency was also similar for pigs in all four treatment groups. Pigs in lot 4 that were fed shelled corn and protein supplement free choice gained slightly slower but they had more efficient gains than pigs in the other three lots. As in trial 1, barrows gained significantly faster than gilts ($P < .01$).

Trials 3 and 4 of experiment 1 were replicates. Average daily gain and feed efficiency were not significantly different between the methods of feeding or levels of vitamins fed. Pigs receiving no supplemental vitamins (lot 1) were less efficient in feed utilization than the other treatment groups, however, the lack of supplemental vitamins did not significantly affect average daily gain. In both trials, pigs fed the medium level of vitamins in the drinking water appeared to utilize their feed more efficiently than pigs in the other treatment groups, but the experimental design did not permit a statistical analysis. Liver and plasma vitamin A were also determined in these trials. Pigs receiving their vitamins in the diet (positive control pigs) had significantly higher liver vitamin A levels than all other treatments ($P<.01$). In addition, the control pigs in trial 4 had significantly higher plasma vitamin A levels ($P<.05$). These data suggest better utilization of the vitamins in the feed or instability or settling out of the vitamins in the water. The data also suggest that the high level of vitamins in the water had no beneficial effect on rate of gain or feed efficiency. However, proper fortification of each nutrient in water is not well established. Extensive research is needed to determine the quantity of nutrients needed per gallon of water and methods of providing an economical premix.

The performance of rats receiving different levels of vitamins in the water was similar to the performance of rats fed the same level of vitamins in the feed. Average daily gain of rats increased with the increase in vitamins in feed or water. The fastest gaining rats were

also the most efficient in feed utilization. Liver vitamin A also appeared to be similar for the two methods of feeding.

In trial 1, rats fed the high level of vitamins in the water had a significantly greater total gain than rats receiving no supplemental vitamins in the water ($P<.01$). They also had a greater total gain than the rats fed the medium level of vitamins in the water ($P<.05$). Rats fed the purified diet without any supplemental vitamins lost weight although their feed consumption was approximately 50% of the intake of rats receiving the high level of vitamins in the drinking water.

Rats fed the low and medium levels of vitamins in the feed had significantly greater total gains than rats receiving no supplemental vitamins in the feed ($P<.01$). The medium level of vitamins in the feed also improved feed utilization for these rats over those receiving no supplemental vitamins in their diet ($P<.05$).

In general, total feed intake was increased when more vitamins were fed. Water consumption also increased with the higher intake of feed and vitamins, but the difference was smaller between treatments than the effect on feed consumption.

The performance of rats in trials 2 and 3 coincide with the performance of rats in trial 1. As the level of vitamins increased in the feed or water, average total gain increased and feed per unit of gain decreased. Rats receiving the medium and high levels of vitamins in the drinking water had greater total gains and more efficient feed utilization than rats receiving no supplemental vitamins in the water

($P < .01$). The high level of vitamins in the water did not produce significantly greater gains than the medium level of vitamins in the water. Rats receiving the medium and high levels of vitamins in the feed had significantly greater total gains than rats receiving no supplemental vitamins in the feed ($P < .05$). Feed utilization was also improved significantly ($P < .01$). The high level of vitamins in the feed did not improve total gain or feed efficiency when compared to the medium level of vitamins in the feed.

Liver vitamin A was also used as criteria to measure performance of rats fed different levels of vitamins in the feed and water. In general, these results did not indicate any significant difference in liver vitamin A levels due to the two methods of feeding. However, rats receiving the high level of vitamins in the water had significantly lower liver vitamin A levels than rats receiving no supplemental vitamins in the drinking water ($P < .05$). The same trend is also observed when the vitamins were supplied through the feed, but the differences were not significant.

Since the microingredients were dispersed in water, there was no problem of thoroughly mixing these ingredients. The pure undiluted vitamins can be added to the water, whereas their addition in the dry ration required premixing and then extensive mixing in the complete ration. The dispersion and stability of vitamins in the water needs extensive research. The B vitamins are theoretically soluble in water and should become dispersed in the solution. Vitamin A and D, which were also added to the drinking water, are not water soluble. However,

water dispersible vitamin A and D were used in these trials. Analysis of water samples to determine the degree of separation (if any) of the nutrients in solution should be determined. Performance of pigs fed supplemental vitamins in the water does not suggest a problem in physical breakdown of the nutrients in solution. Data, however, from the vitamin A analysis did indicate higher liver vitamin A levels for pigs fed a completely mixed air-dry diet.

The practicality of this method of feeding is speculative since research of this type has not been conducted extensively. Acceptance of this method of feeding will depend on the advantages provided to the swine producer.

LITERATURE CITED

Almquist, H. J. 1952. Relation of vitamin A intake to plasma liver vitamin A content. Arch. Biochem. Biophys. 39:243.

A.O.A.C. 1960. Official Methods of Analysis (9th ed.). Association of Official Chemists. Washington, D. C.

Barnhart, C. E., D. V. Catron, G. C. Ashton and L. Y. Quinn. 1957. Effects of dietary pantothenic acid levels on the weanling pig. J. Animal Sci. 16:396.

Becker, D. E., J. R. Mitchell, Jr., A. H. Jensen, S. W. Terrill and H. W. Norton. 1963. Nicotinic acid-tryptophan relationship in the nutrition of the weanling pig. J. Animal Sci. 22:1117. (Abstr.).

Bethke, R. M., Wise Burroughs, O. H. M. Wilder, B. H. Edgington and W. L. Robison. 1946. The comparative efficacy of vitamin D from irradiated yeast and cod-liver oil for growing pigs, with observations on their vitamin D requirements. Ohio Agr. Exp. Sta. Bul. 667.

Braude, R., A. S. Foot, K. M. Henry, S. K. Kon and S. Y. Thompson. 1941. Vitamin A studies with rats and pigs. Biochem. J. 35:693.

Braude, R. and S. K. Kon. 1946. Observations on the nicotinic acid requirements of pigs. Biochem. J. 40:843.

Cartwright, G. E., B. Tatting and M. W. Wintrobe. 1948. Niacin deficiency anemia in swine. Arch. Biochem. 19:109.

Catron, D. V., R. W. Bennison, H. M. Maddock, G. C. Ashton and P. G. Homeyer. 1953. Effects of certain antibiotics and vitamin B_{12} on pantothenic acid requirements of growing and finishing swine. J. Animal Sci. 12:51.

Catron, D. V., D. Richardson, L. A. Underkofler, H. M. Maddock and C. W. Friedland. 1952. Vitamin B_{12} requirements of weanling pigs. J. Nutr. 47:461.

Christensen, N. O., R. Elkjaer, A. Engelund, T. Munch and P. Terp. 1958. Studies on the absorption of vitamin A. 2. Experiments on liver storage in rats and pigs. Nutr. Abstr. and Rev. 28:1052.

Clayton, C. C. and C. A. Baumann. 1944. Vitamin A storage and factors that affect the liver. J. Nutr. 27:155.

Cunha, T. J. 1957. Swine Feeding and Nutrition. Interscience Publishers, New York, New York. p. 90.

Dunlop, G. 1934. Paralysis and avitaminosis in swine. J. Agr. Sci. 24:435.

Ellis, N. R. 1946. The vitamin requirements of swine. Nutr. Abstr. and Rev. 16:1.

Ellis, N. R. and L. L. Madsen. 1944. The thiamine requirement of pigs as related to the fat content of the diet. J. Nutr. 27:253.

Ellis, N. R., L. L. Madsen and C. O. Miller. 1943. Pantothenic acid and pyridoxine as factors in the occurrence of locomotor incoordination in swine. J. Animal Sci. 2:365. (Abstr.).

Farris and Griffith. 1962. The Rat in Laboratory Investigation. Hafner Publishing, New York, New York.

Frape, D. L., V. C. Speer, V. W. Hays and D. V. Catron. 1959. The vitamin A requirement of the young pig. J. Nutr. 68:173.

Ganguly, J. 1960. Vitamin A storage in blood and liver. Vitamins and Hormones 18:392.

Ganguly, J. and Krinsky. 1953. Absence of relationship between vitamin A alcohol levels in plasma and in liver of rats. Biochem. J. 54:177.

Glover, J., T. W. Goodwin and R. A. Morton. 1947. Studies in vitamin A. The relationship between blood vitamin A levels and liver stores in rats. Biochem. J. 41:97.

Golding, J. and A. S. Foot. 1935. Avitaminosis A in pigs. Agr. Progress 12:123.

Gorodeckji, A. A. 1963. Effektivnost podkormki porosjat holinom i vitaminom B_{12} (Effectiveness of supplements of choline and vitamin B_{12} for young pigs). Zivolnovodstov 5:78.

Grifo, A. P., Jr., H. S. Teague and B. A. Dehority. 1964. Vitamin B_{12} for growing-finishing pigs. J. Animal Sci. 23:771.

Guerrant, N. B. 1949. Influence of age and of vitamin A intake on the storage of vitamin A in the liver of the rat. J. Nutr. 37:37.

Guilbert, H. R., C. E. Howell and G. H. Hart. 1940. Minimum vitamin A and carotene requirements of mammalian species. J. Nutr. 19:91.

Halpern, G. R., J. Biely and F. Hardy. 1947. Utilization of vitamin A in water emulsion. Science 106:40.

Heinemann, W. W., M. E. Ensminger, T. J. Cunha and E. C. McCullock. 1946. The relation of the amount of thiamine in the ration of the hog to the thiamine and riboflavin content of the tissue. J. Nutr. 31:107.

Hentges, J. F., Jr., R. H. Grummer, P. H. Phillips and G. Bohstedt. 1952. The minimum requirement of young pigs for a purified source of carotene. J. Animal Sci. 11:266.

High, E. G. and S. S. Wilson. 1956. Studies on vitamin A ester: alcohol partition between the liver, kidneys and plasma of rats. Arch. Biochem. Biophys. 62:163.

Hjarde, W., A. Neimenn-Sorensen, B. Palludan and P. H. Sorenson. 1961. Investigations concerning vitamin A requirements, utilization and deficiency symptoms in pigs. Acta Agr. Scandinavica 11:13.

Hoefer, J. A. 1963. Feeding methods for swine. Mich. Agr. Exp. Sta. Swine Field Day Rpt.

Hopkins, F. G. 1906. Quoted from Maynard, L. A. and J. K. Loosli. 1962. Animal Nutrition (5th ed.). McGraw-Hill Book Co., New York, New York.

Hughes, E. H. 1939. The role of riboflavin and other factors of the vitamin B complex in the nutrition of the pig. J. Nutr. 17:527.

Hughes, E. H. 1940. The minimum requirement of thiamine for the growing pig. J. Nutr. 20:239.

Hughes, E. H. 1943. The minimum requirement of nicotinic acid for the growing pig. J. Animal Sci. 2:23.

Hughes, E. H. and N. R. Ittner. 1942. The minimum requirement of pantothenic acid for the growing pig. J. Animal Sci. 1:116.

Hutchinson, H. D., S. W. Terrill, A. H. Jensen, D. E. Becker and H. W. Norton. 1957. Comparison of free choice and complete rations for growing and finishing pigs on pasture and drylot. J. Animal Sci. 16:562.

Johnson, R. M. and C. A. Baumann. 1947. Storage and distribution of vitamin A in rats fed certain isomers of carotene. Arch. Biochem. 14:361.

Johnson, R. M. and C. A. Baumann. 1948. Relative significance of growth and metabolic rate upon the utilization of vitamin A by the rat. J. Nutr. 35:703.

Johnson, B. C. and M. F. James. 1948. Choline deficiency in the baby pig. J. Nutr. 36:339.

Johnson, D. W. and L. S. Palmer. 1939. Individual and breed variations in pigs on rations devoid of vitamin D. J. Agr. Res. 58:929.

Kieckebush, W. 1960. Vitamin A-Wachstums- und Leber speichertest bei Rutten, mit gleichzeitiger PruFung eines wasser-und fettloslichen Praparates (Growth and liver storage tests for vitamin A with rats, with simultaneous testing of a water- and a fat-soluble preparation). Ztschr. Ernahrungswiss 1:221.

Krider, J. L., S. W. Terrill and R. F. Van Poucke. 1949. Response of weanling pigs to various levels of riboflavin. J. Animal Sci. 8:121.

Lemley, J. M., R. A. Brown, O. D. Bird and A. D. Emmett. 1947. Absorption and storage of vitamin A in the liver of the rat. J. Nutr. 33:53.

Luecke, R. W., J. A. Hoefer and F. Thorp, Jr. 1953. The supplementary effects of calcium pantothenate and aureomycin in a low protein ration for weanling pigs. J. Animal Sci. 12:605.

Luecke, R. W., E. T. Komegay, E. R. Miller and J. A. Hoefer. 1961. Effect of either supplementary zinc or vitamin D in swine rations varying in calcium level. J. Animal Sci. 20:942. (Abstr.).

Luecke, R. W., W. N. McMillen and F. Thorpe, Jr. 1950. Further studies of P.A. deficiency in weanling pigs. J. Animal Sci. 9:78.

Luecke, R. W., W. N. McMillen, F. Thorp, Jr. and C. Tull. 1947. The relationship of nicotinic acid, tryptophan and protein in the nutrition of the pig. J. Nutr. 33:251.

Luecke, R. W., W. N. McMillen, F. Thorp, Jr. and C. Tull. 1948. Further studies on the relationship of nicotinic acid, tryptophan and protein in the nutrition of the pig. J. Nutr. 36:417.

McCollum, E. V. and M. Davis. 1913. Necessity of certain lipins in the diet during growth. J. Biol. Chem. 15:167.

McMillen, W. N., R. W. Luecke and F. Thorp, Jr. 1949. The effect of liberal B vitamin supplementation on growth of weanling pigs fed rations containing a variety of feedstuffs. J. Animal Sci. 8:518.

Miller, E. R., D. A. Schmidt, J. A. Hoefer and R. W. Luecke. 1955. The thiamine requirement of the baby pig. J. Nutr. 56:423.

Miller, E. R., D. E. Ullrey, C. L. Zutaut, J. A. Hoefer and R. W. Luecke. 1965. Mineral balance studies with baby pigs: Effects of dietary vitamin D_2 level upon Ca, P and Mg balance. J. Nutr. 85:255.

Miller, E. R., D. E. Ullrey, C. L. Zutaut, J. A. Hoefer and R. W. Luecke. 1965. Comparison of casein and soy proteins upon mineral balance and vitamin D_2 requirements of the baby pig. J. Nutr. 85:347.

Mitchell, H. H., B. C. Johnson, T. L. Hamilton and W. T. Haines. 1950. The riboflavin requirement of the growing pig at two environmental temperatures. J. Nutr. 41:317.

Moore, T. 1957. Vitamin A in Mammals. Elsevier Publishing Co. p. 143.

Morrison, F. B., G. Bohstedt and J. M. Fargo. 1919. New Farm Facts: Yellow versus white corn for pigs. Wisc. Agr. Exp. Sta. Annual Rpt. Bul. 323:10.

Myers, G. S., Jr., H. D. Eaton and J. E. Rouseau, Jr. 1959. Relative value of carotene from alfalfa and vitamin A from a dry carrier fed to lambs and pigs. J. Animal Sci. 18:288.

Nelson, E. C., B. A. Dehority, H. S. Teague, V. L. Sanger and W. D. Pounden. 1962. Effect of vitamin A intake on some biochemical and physiological changes in swine. J. Nutr. 76:325.

Nelson, V. E., A. R. Lamb and V. G. Heller. 1922. The vitamin requirements of various species of animals III. Am. J. Physiol. 59:335.

Nesheim, R. O. and B. C. Johnson. 1950. Effect of a high level of methionine on the dietary choline requirement of the baby pig. J. Nutr. 41:149.

N.R.C. 1959. Nutrient Requirements of Domestic Animals, No. 2. Nutrient Requirements of Swine. National Research Council, Washington, D. C.

Powick, W. C., N. R. Ellis and C. N. Dale. 1948. Relationship of tryptophane to nicotinic acid in the feeding of growing pigs. J. Animal Sci. 7:228.

Richardson, D., D. V. Catron, L. A. Underkofler, H. M. Maddock and W. C. Friedland. 1951. Vitamin B_{12} requirements of male weanling pigs. J. Nutr. 44:371.

Smith, W. H., R. A. Pickett, J. H. Conrad and W. M. Beeson. 1961. Effect of vitamin A supplementation on a corn-soy ration for swine. J. Animal Sci. 20:952. (Abstr.).

Sorbel, A. E., M. Sherman, J. Lichtblaw, S. Snow and B. Kramer. 1948. Comparison of vitamin A liver storage following administration of vitamin A in oily and aqueous media. J. Nutr. 35:225.

Sorbel, A. E. 1952. The problem of absorption and transportation of fat-soluble vitamins. Vitamins and Hormones 10:47.

Squibb, R. L., J. E. Braham and N. S. Scrimshaw. 1956. Absorption of vitamin A in aqueous and oil solutions by depleted New Hampshire chicks. Poul. Sci. 35:73.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York, New York.

Stothers, S. C., D. A. Schmidt, R. L. Johnston, J. A. Hoefer and R. W. Luecke. 1955. The P.A. requirements of the baby pig. J. Nutr. 57:47.

Terrill, S. W., C. B. Ammerman, D. E. Walker, R. M. Edwards, H. W. Norton and D. E. Becker. 1955. Riboflavin studies with pigs. J. Animal Sci. 14:593.

Ullrey, D. E., E. R. Miller, R. D. Struthers, R. E. Peterson, J. A. Hoefer and H. H. Hall. 1965. Vitamin A activity of fermentation B-carotene for swine. J. Nutr. 85:375.

Vohs, R. L., H. M. Maddock, D. V. Catron and C. C. Culbertson. 1951. Vitamin B_{12}, APF concentrate and dried whey for growing-fattening pigs. J. Animal Sci. 10:42.

Wahlstrom, R. C. and D. E. Stolte. 1958. The effect of supplemental vitamin D in rations for pigs fed in the absence of direct sunlight. J. Animal Sci. 17:699.

Wise, G. W., N. L. Jacobson, R. S. Allen and S. P. Yang. 1958. Rate of absorption of carotene and of vitamin A from the alimentary tract of dairy calves. II. Effects of methods of dispersion and of administration. J. Dairy Sci. 41:143.

Young, E. P. and F. C. Wingert. 1959. Free choice versus complete mixed rations for growing and finishing swine in drylot. Maryland Agr. Exp. Sta. Swine Field Day Rpt.

APPENDIX

TABLE 1. ANALYSIS OF VARIANCE FOR AVERAGE DAILY GAIN, EXPERIMENT 1, TRIAL 1

Source of variation	d.f.	M.S.
Total	23	
Treatment (T)	2	0.213228**
Sex (S)	1	0.053110**
T x S	2	0.003371
Error	18	0.004774

** Significant difference in treatment ($P<.01$).
** Barrows gained significantly faster than gilts ($P<.01$).

TABLE 2. ANALYSIS OF VARIANCE FOR AVERAGE DAILY GAIN, EXPERIMENT 1, TRIAL 2

Source of variation	d.f.	M.S.
Total	38	
Treatment (T)	3	59.7603
Sex (S)	1	451.9023**
Breed (B)	1	68.5358
T x S	3	19.4403
T x B	3	49.0693
S x B	1	30.3512
Error	26	54.0511

** Barrows gained significantly faster than gilts ($P<.01$).

TABLE 3. ANALYSIS OF VARIANCE FOR AVERAGE DAILY GAIN, EXPERIMENT 1, TRIAL 3

Source of variation	d.f.	M.S.
Total	38	
Treatment (T)	4	40.3018
Sex (S)	1	917.7591**
T x S	4	42.9085
Error	29	47.6325

** Barrows gained significantly faster than gilts ($P<.01$).

TABLE 4. ANALYSIS OF VARIANCE FOR AVERAGE DAILY GAIN, EXPERIMENT 1, TRIAL 4

Source of variation	d.f.	M.S.
Total	39	
Treatment (T)	4	11.89
Sex (S)	1	1.12
Breed (B)	1	1.81
T x S	4	1.84
T x B	4	4.38
S x B	1	0.04
T x S x B	4	13.22
Error	20	10.45

TABLE 5. ANALYSIS OF VARIANCE FOR PLASMA AND LIVER VITAMIN A, EXPERIMENT 1, TRIALS 3 AND 4

Source of variation	d.f.		M.S.	
	Plasma	Liver	Plasma	Liver
		Trial 3		
Total	17	17		
Treatment	4	4	15403.121	10454.016**
Error	13	13	11079.823	98.762
		Trial 4		
Total	15	19		
Treatment (T)	4	4	35.1598*	19551.25 **
Breed (B)	1	1	25.3125	59.52
T x B	3[a]	4	4.4313	311.62
Error	7	10	6.9108	342.62

[a] One treatment was represented by only one breed.
* ($P<.05$).
** ($P<.05$).

TABLE 6. ANALYSIS OF VARIANCE FOR TOTAL GAIN, EXPERIMENT 2, TRIAL 1

Source of variation	d.f.	M.S.
Total	59	
Water (W)	3	6397.82**
Feed (F)	2	38636.25**
W x F	6	1121.12
Error	48	618.22

** Significant difference in total gain for feed and water treated rats due to different vitamin intakes ($P<.01$).

TABLE 7. ANALYSIS OF VARIANCE FOR FEED EFFICIENCY, EXPERIMENT 2, TRIAL 1

Source of variation	d.f.	M.S.
Total	54	
Feed (F)	2	3226.1142*
Water (W)	3	1336.6552
F x W	6	1202.5735
Error	43	934.5513

* Significant difference in feed efficiency due to vitamin level in feed ($P<.05$).

TABLE 8. ANALYSIS OF VARIANCE FOR TOTAL GAIN, EXPERIMENT 2, TRIALS 2 AND 3

Source of variation	d.f.	M.S.
Total	95	
Replicate (R)	1	2002.03*
Water (W)	3	20447.05**
Feed (F)	3	15159.01*
R x W	3	703.25
R x F	3	971.85*
W x F	9	2672.22**
R x W x F	9	671.73*
Error	64	296.84

* ($P<.05$).
** ($P<.01$).

TABLE 9. ANALYSIS OF VARIANCE FOR FEED EFFICIENCY, EXPERIMENT 2, TRIALS 2 AND 3

Source of variation	d.f.	M.S.
Total	92	
Replicate (R)	1	27.6083
Water (W)	3	617.8759**
Feed (F)	3	708.0731**
R x W	3	11.0833
R x F	3	15.1273
W x F	9	305.8307**
Error	70	32.4966

** ($P < .01$).

TABLE 10. ANALYSIS OF VARIANCE FOR LIVER VITAMIN A, EXPERIMENT 2, TRIALS 2 AND 3

Source of variation	d.f.	M.S.
Total	91	
Water (W)	3	66.4525*
Feed (F)	3	35.0486
Replicate (R)	1	230.5991**
W x F	9	24.9234
W x R	3	49.1801*
F x R	3	28.9294
W x F x R	9	8.3825
Error	60	14.7362

* ($P < .05$).
** ($P < .01$).